Human CtIP: A 'double agent' in DNA repair and tumorigenesis

Mozaffari, Nour L ; Pagliarulo, Fabio ; Sartori, Alessandro A

Abstract: Human CtIP was originally identified as an interactor of the retinoblastoma protein and BRCA1, two bona fide tumour suppressors frequently mutated in cancer. CtIP is renowned for its role in the resection of DNA double-strand breaks (DSBs) during homologous recombination, a largely error-free DNA repair pathway crucial in maintaining genome integrity. However, CtIP-dependent DNA end resection is equally accountable for alternative end-joining, a mutagenic DSB repair mechanism implicated in oncogenic chromosomal translocations. In addition, CtIP contributes to transcriptional regulation of G1/S transition, DNA damage checkpoint signalling, and replication fork protection pathways. In this review, we present a perspective on the current state of knowledge regarding the tumour-suppressive and oncogenic properties of CtIP and provide an overview of their relevance for cancer development, progression, and therapy.

DOI: https://doi.org/10.1016/j.semcdb.2020.09.001

Seminars in Cell and Developmental Biology xxx (xxxx) xxx

Contents lists available at ScienceDirect

Seminars in Cell and Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

Human CtIP: A 'double agent' in DNA repair and tumorigenesis

Nour L. Mozaffari [1], Fabio Pagliarulo [1], Alessandro A. Sartori *

Institute of Molecular Cancer Research, University of Zurich, Zurich, Switzerland

ARTICLE INFO

Keywords:
CtIP/RBBP8
BRCA1
DNA end resection
double-strand break repair
replication stress
genome instability
pancreatic cancer
synthetic lethality
transcriptional regulation
tumour suppressor

ABSTRACT

Human CtIP was originally identified as an interactor of the retinoblastoma protein and BRCA1, two *bona fide* tumour suppressors frequently mutated in cancer. CtIP is renowned for its role in the resection of DNA double-strand breaks (DSBs) during homologous recombination, a largely error-free DNA repair pathway crucial in maintaining genome integrity. However, CtIP-dependent DNA end resection is equally accountable for alternative end-joining, a mutagenic DSB repair mechanism implicated in oncogenic chromosomal translocations. In addition, CtIP contributes to transcriptional regulation of G1/S transition, DNA damage checkpoint signalling, and replication fork protection pathways. In this review, we present a perspective on the current state of knowledge regarding the tumour-suppressive and oncogenic properties of CtIP and provide an overview of their relevance for cancer development, progression, and therapy.

1. Introduction

Human CtIP is an 897-amino acid nuclear adaptor protein with multiple roles in DNA metabolism and genome stability [1,2]. Initially thought to function exclusively as a regulator of gene expression, continued research has revealed that CtIP plays a significant role in DNA double-strand break (DSB) repair pathway choice, with opposing consequences for genome stability and tumorigenesis. Herein, we review the current literature on CtIP's molecular and biological activities with a particular focus on their clinical relevance for cancer progression and treatment outcomes.

1.1. CtIP: History at a glance

Human CtIP was first isolated in 1998 using yeast two-hybrid screening experiments performed in four independent laboratories. Schaeper et al. identified CtIP as an interacting protein of the oncogenic transcriptional corepressor CtBP. Of note, CtIP binds CtBP through a PLDLS motif conserved among E1A proteins of human adenoviruses [3]. Fusco et al. called the same polypeptide RIM (encoded by *RBBP8* located at 18q11.2) by virtue of its ability to interact with the Rb tumour suppressor protein via a conserved LxCxE Rb-binding motif [4]. Shortly thereafter, two studies reported CtIP to specifically interact with the BRCT domain of BRCA1 [5,6]. Together, these early findings implicated CtIP in suppressing tumorigenesis, possibly by regulating the transcription of cell cycle-related genes [7].

To date, orthologs of human CtIP have been characterised in several uni- and multicellular organisms, including *S. cerevisiae* (Sae2/Com1), *S. pombe* (Ctp1), *P. tetraurelia* (PtCtIP), *C. elegans* (COM-1), and *A. thaliana* (AtCOM1). Remarkably, CtIP deficiency results in impaired repair of DSBs in somatic cells and during meiosis [2,8–11]. It is now well established that CtIP associates with the MRE11-RAD50-NBS1 (MRN) nuclease complex to initiate 5'-3' resection of broken DNA ends, a critical determinant of DSB repair pathway choice [12–14].

1.2. Architecture and Protein Interaction Network of CtIP

CtIP is largely intrinsically disordered with two conserved domains located at the N- and C-terminus of the protein (Fig. 1) [1]. The N-terminal domain is comprised of coiled-coil heptad repeats mediating CtIP dimerisation [15,16]. In addition, a short α-helical motif preceding the coiled-coil region promotes CtIP tetramerisation [17–19]. The C-terminal Sae2-like domain harbours a highly conserved DNA binding motif, which enables CtIP to form protein-DNA filaments and promote DNA bridging [18–20]. Two independent studies reported that CtIP exhibits intrinsic structure-specific endonuclease activity mediated by two distinct catalytic sites, a topic that is still controversially discussed in the literature [2,1,18,21–23].

* Corresponding author.
E-mail address: sartori@imcr.uzh.ch (A.A. Sartori).
[1] Equal contribution

https://doi.org/10.1016/j.semcdb.2020.09.001
Received 21 April 2020; Received in revised form 20 July 2020; Accepted 2 September 2020

Typical for multivalent adaptor proteins, CtIP contains various short linear sequence motifs facilitating interactions between protein-binding partners, some of which are governed by CtIP phosphorylation (Fig. 1) [2,24]. For instance, CDK-mediated phosphorylation of CtIP at S327 is a prerequisite for the binding of BRCA1 BRCT domains and has been proposed to speed up DSB resection in S/G2-cells [25,26]. In contrast, CDK-dependent phosphorylation of CtIP at T847 is indispensable for DNA end resection, most likely by fostering MRN-CtIP complex formation [23,27]. Two additional CDK phosphorylation sites in CtIP, S276 and T315, are required for the binding of PIN1, a unique peptidyl-prolyl *cis-trans* isomerase found to be overexpressed in many cancers and to promote uncontrolled cell proliferation [28–30]. CtIP isomerisation has been proposed to facilitate CUL3-KLHL15-mediated CtIP ubiquitination and subsequent proteasomal degradation to limit CtIP's resection activity [28,31]. APC/C-Cdh1 recognizes CtIP through a conserved KEN box motif and targets CtIP for ubiquitin-proteasome-mediated degradation in G2, presumably to prevent inappropriate DNA resection in mitosis [32]. Following genotoxic insults, CtIP phosphorylation at T859 by the apical DNA damage response (DDR) kinases ATM and ATR is important for stable chromatin association of CtIP and efficient DSB processing [33,34]. Remarkably, a recent biochemical study reported that CtIP phosphorylation on T847 and T859 is critical for MRN endonuclease-mediated removal of DNA-PK from DNA ends to allow resection and repair of DSBs by HR [35]. CtIP also directly associates with FANCD2, the central mediator of the Fanconi Anemia tumour suppressor pathway responsible for the repair of DNA interstrand crosslinks [36,37]. Finally, CtIP structural interaction with the breast cancer oncogene LMO4 has been hypothesised to sequester CtIP away from DNA repair complexes [38].

CtIP expression levels are tightly regulated throughout the cell cycle, remaining low in G1 and increasing in S and G2 phases, when sister chromatids are available as a template for HR repair [1,8]. Besides the aforementioned mechanisms controlling CtIP protein turnover by ubiquitin-proteasomal processing, there is accumulating evidence that CtIP abundance is also regulated at the level of transcription. Liu et al. showed that CtIP can bind to its own promoter, upregulating its expression during G1/S progression (Fig. 2) [39]. Moreover, several microRNAs have been shown to silence CtIP expression. Martin et al. reported that miR-335-mediated suppression of CtIP is released following ionising radiation (IR)-induced ATM activation, classifying miR-335 as a potential radiosensitiser for clinical use [40,41]. Similarly, CtIP transcripts are negatively regulated by miR-19, a member of the miR-17~92 cluster whose expression is upregulated in many cancer types [42,43]. More recently, downregulation of CtIP by miR223-3p was identified as a protective mechanism against chromosomal rearrangements in developing haematopoietic cells [44].

2. Versatile Roles of CtIP in Genome Maintenance

CtIP's ability to specifically interact with multiple binding partners (Fig. 1) correlates well with its function in diverse genome maintenance mechanisms, including cell cycle control, DNA damage repair, and DNA replication (Fig. 2) [1,2,8].

2.1. Transcriptional regulation and cell cycle checkpoints

Early studies connecting the CtIP-CtBP corepressor complex to Rb family and BRCA1 proteins suggested a prominent role of CtIP in transcriptional regulation of cell cycle events (Fig. 2) [2]. For instance, the interaction between CtIP and Rb has been shown to alleviate Rb-mediated transcriptional repression of certain E2F target genes (e.g. *CCND1*), thus facilitating G1/S transition [39,45]. Conversely, in response to DNA damage, CtIP was reported to bind to the promoter and induce the expression of the CDK inhibitor *p21*, hence blocking G1/S transition [46]. Moreover, binding of CtIP-CtBP to BRCA1 has been implicated in preventing *p21* promoter transactivation by BRCA1, while DNA damage-induced disruption of the BRCA1-CtIP interaction could relieve *p21* and *GADD45A* transcriptional repression [47,48]. Similarly, association of CtIP with BRCA1 and the BRCA1-binding transcriptional repressor ZBRK1 negatively regulates the expression of pro-tumorigenic factors such as *ANG1* and *HMGA2*, and loss of this heterotrimeric complex has been shown to enhance mammary tumorigenesis [49,50]. In addition, binding of the CtIP-LMO4 complex to BRCA1 inhibits BRCA1-mediated transcriptional activation [51]. Consequently, CtIP appears to exert dual control over gene expression and promoter activities depending on its binding partners.

In contrast, Yu and Chen first demonstrated that formation of the CtIP-BRCA1 complex in G2 is required for the IR-induced phosphorylation of Chk1 and activation of the G2/M transition checkpoint [25]. In agreement with these findings, it was recently shown that CtIP promotes G2/M arrest through ATR-Chk1 activation rather than by induction of the p53-p21/GADD45a pathway [52]. ATR-Chk1 signalling in response to DNA damage is initiated by the recruitment of ATR to RPA-coated ssDNA tracks, key intermediates formed during DSB resection (Fig. 2) [53]. Therefore, while CtIP regulates G1/S transition mostly at the level of transcription, its G2/M checkpoint function is primarily mediated by DNA end resection.

2.2. DNA end resection and DSB repair pathway choice

In a process commonly referred to as 'DNA end resection', CtIP associates with the MRN complex to promote limited (5'-3') nucleolytic processing of DSBs, which commits cells to homology-directed repair mechanisms (HR, SSA, and a-EJ) and prevents repair by c-NHEJ (Fig. 2)

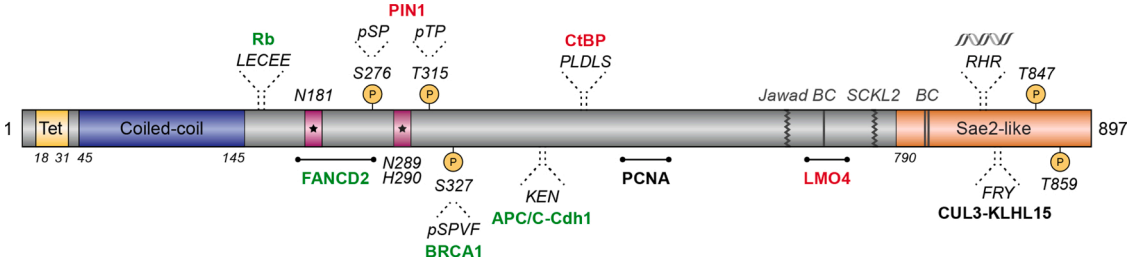

Fig. 1. Human CtIP, a polyvalent adaptor protein.
CtIP is composed of a tetramerisation motif (Tet) and an extended coiled-coil region at the N-terminus, an unstructured central region and an Sae2-like domain at the C-terminus. Two non-canonical nuclease motifs harbouring residues implicated in catalytic activity are highlighted by asterisks. Short linear sequence motifs promoting specific protein interactions and DNA binding are indicated by dashed lines. CtIP interactors classified as tumour suppressors and oncogenes are highlighted in green and red letters, respectively. Selected serine/threonine (S/T) phosphorylation sites are indicated. CtIP C-terminal truncation variants identified in Jawad and Seckel (*SCKL2*) syndrome patients [83] are indicated by jagged lines. Three *RBBP8* mutations (Q643P, E804Δ and R805G) associated with early-onset breast cancer (*BC*) are indicated by straight lines [78].

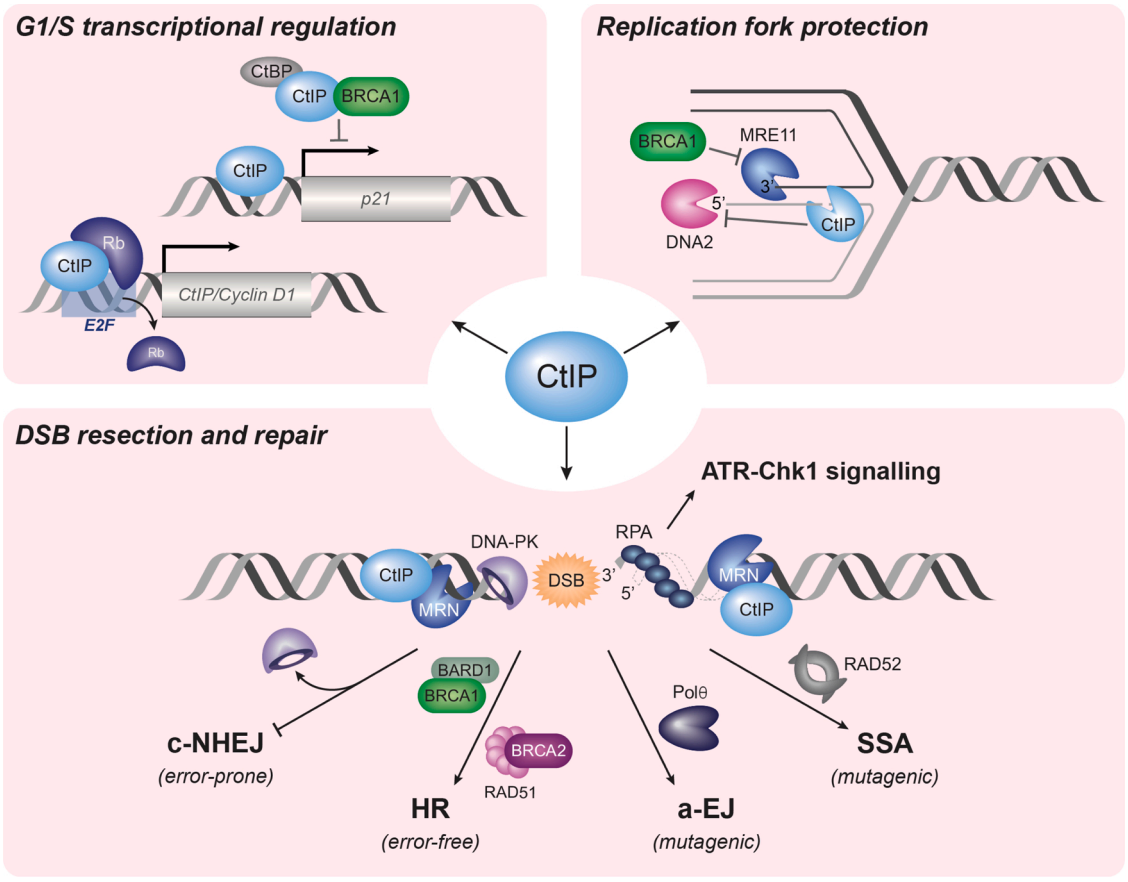

Fig. 2. CtIP at the crossroads of multiple genome maintenance mechanisms.
Upper left, CtIP is implicated in the transcriptional regulation of several genes during G1/S transition. Upon interaction with CtIP, Rb is released from E2F-responsive promoters, where CtIP binds and activates gene expression. Under normal conditions, *p21* is repressed by BRCA1-CtIP-CtBP. DNA damage disrupts this complex and induces the recruitment of CtIP to *p21* promoter elements, enhancing *p21* expression. ***Middle,*** In response to DSBs, CtIP associates with the MRN complex and stimulates MRE11 endonuclease activity to initiate bidirectional DNA end resection, using EXO1 or BLM/DNA2 in the 5'-3' direction away from the DSB, and MRE11's exonuclease in the 3'-5' direction towards the DSB end (not shown). In addition, CtIP/MRN-mediated endonucleolytic cleavage displaces DNA-PK (composed of the Ku70-80 heterodimer and DNA-PKcs) from broken DNA ends. These processes effectively suppress c-NHEJ. Ensuing resection of the 5' strand leads to the exposure of 3'-ssDNA overhangs, which commits DSB repair to homology-directed pathways (HR, a-EJ or SSA) and activates ATR-Chk1 signalling. HR relies on the sequential actions of BRCA1-BARD1 and BRCA2 to direct RAD51-dependent homologous DNA template search and ensure accurate DSB repair. In the absence of HR or when HR repair is inappropriate due to the lack of a sister chromatid template, mutagenic repair pathways such as Polθ-dependent a-EJ or RAD52-mediated SSA are activated. ***Upper right,*** Following DNA replication stress, CtIP is recruited to stalled replication forks, where it inhibits DNA2-dependent over-resection of nascent DNA, presumably via endonucleolytic incision of the reversed 5'-strand. Therein, CtIP acts synergistically with BRCA1, which protects reversed forks from MRE11-mediated degradation.

[12,14,54,55]. However, while HR faithfully restores the original DNA sequence at the break site in most cases, SSA and a-EJ frequently result in deletions and are associated with pathological chromosome rearrangements [14]. Importantly, BRCA1, in complex with its constitutive partner BARD1, counteracts 53BP1 chromatin accumulation to facilitate CtIP-mediated resection, thereby tipping the balance towards the exclusion of c-NHEJ and induction of HR, especially for breaks encountered during DNA replication [56,57]. Extended resection is carried out by dedicated DNA nucleases and helicases, most notably EXO1, DNA2, and BLM [13]. Interestingly, CtIP restrains EXO1 exonuclease activity but accelerates BLM/DNA2-mediated DNA cleavage, presumably via direct protein-protein interactions, thus coordinating both short- and long-range resection processes [60–62]. Resulting 3'-ssDNA overhangs are rapidly coated by the heterotrimeric RPA complex, creating a platform for ATR-Chk1 signalling (Fig. 2) [63]. The choice between error-free HR and mutagenic a-EJ and SSA pathways mainly happens downstream of DSB resection [14,64,65]. In the presence of a homologous DNA template, preferentially the intact sister chromatid, BRCA2 mediates RAD51 filament assembly on ssDNA to promote subsequent strand invasion and exchange steps of HR. When HR is inappropriate, exposure of microhomologies between resected DNA arms can drive RPA removal and gap-filling synthesis by Polθ associated with a-EJ. Alternatively, RAD52-mediated annealing of complementary ssDNA ends between repeated sequences occurs during SSA. Remarkably, DSB resection and repair activities of CtIP have also been reported outside S/G2 phases of the cell cycle. For instance, Löbrich and colleagues could demonstrate that resection-dependent c-NHEJ of IR-induced DSBs in G1-cells contributes to chromosomal translocations and is induced by polo-like kinase 3 (PLK3)-dependent CtIP-S327 phosphorylation and interaction between CtIP and BRCA1 [66,67].

In summary, CtIP-mediated DNA end resection is mostly required for accurate repair of DSBs by HR, but also allows SSA, a-EJ and c-NHEJ to create mutagenic repair products, ranging from simple deletions to complex chromosomal rearrangements.

2.3. DNA replication stress

There is substantial evidence that besides its fundamental role in DSB resection, CtIP is engaged in different processes safeguarding genome

integrity in response to DNA replication stress [68,69]. Cortez and colleagues repeatedly found CtIP to be enriched at active replication forks [70,71], presumably through its direct interaction with PCNA (Fig. 1) [72]. Yeo et al. reported that CtIP binding to FANCD2 facilitates CtIP relocation to damaged replication forks, thereby promoting fork restart and preventing new origin firing during fork recovery [73]. The Hendrickson laboratory showed that CtIP maintains telomere integrity during DNA replication via a similar mechanism [74]. The role of CtIP at stalled forks following hydroxyurea (HU)-induced replication stress is subject to controversy. Initially, it was shown that CtIP promotes the degradation of stalled forks by stimulating MRE11 and EXO1 nuclease activities [75,76]. Our group later demonstrated that CtIP protects reversed forks from nucleolytic degradation by DNA2 (Fig. 2) [77], a finding that was independently confirmed by a study from the Sørensen group [78]. However, while our data implicated CtIP's endonuclease activity in fork protection, Zarrizi et al. proposed that CtIP suppresses fork degradation by counteracting FBH1-mediated removal of RAD51 from stalled forks [77,78]. In addition, we found that CtIP-dependent stimulation of MRE11 endonuclease activity (via pT847) and CtIP-BRCA1 interaction (via pS327) are largely dispensable for fork protection [77]. Of note, recent data suggest that CtIP-BRCA1 interaction is critical for the initation of transcription-associated HR repair of DSBs arising at stalled replication sites upon formation of DNA-RNA hybrids, also termed R-loops [79]. Remarkably, efficient resolution of R-loops was shown to rely on CtIP's endonuclease activity [80]. Recent work from the Morris laboratory established that PIN1-induced conformational change of the BRCA1-BARD1 complex is required for fork protection, but not for HR repair. On this basis, it would be interesting to determine whether a similar separation-of-function mechanism applies to CtIP, an established PIN1 target [28,81]. Interestingly, we could further reveal that CtIP acts in a synergistic or additive manner with BRCA1 in protecting nascent DNA strands from nucleolytic degradation, which potentially contributes to the observed synthetic sick/lethal relationship between CtIP and BRCA1 [77]. Together, these findings highlight an important HR-independent function of CtIP in maintaining genome stability.

3. CtIP's dual role in tumorigenesis and cancer progression

Deregulation of the cell cycle and high levels of genomic instability are not only characteristics of cancer but also a driving force of tumorigenesis [82]. Therefore, CtIP-ascribed molecular activities have mostly been implicated in tumour suppression. This general assumption has gained further support by an early genetic study in mice showing that homozygous deletion of *Ctip* causes early embryonic lethality, while *Ctip*$^{+/-}$ heterozygosity renders mice prone to malignancy, most likely due to CtIP haploid insufficiency [45]. This paradigm has recently been challenged by several studies indicating that CtIP has oncogenic potential facilitating tumorigenesis.

3.1. CtIP mutations and genomic alterations in human disease

Given that *Ctip* is indispensable for viability in mice [45,58], it is not surprising that complete loss-of-function mutations in *CtIP/RBBP8* have not been reported in man. Rare, biallelic hypomorphic *CtIP* variants translating into C-terminal truncated proteins cause Jawad and Seckel syndromes (Fig. 1), genetically heterogeneous conditions characterised by severe microcephaly and mental retardation, but no overt cancer predisposition [83]. Interestingly, Seckel patient-derived cell lines commonly display defects in ATR-dependent DNA damage signalling, certainly a well-established phenotype of CtIP-deficient cells [84,85].

Germline mutations in *BRCA1* or *BRCA2* greatly increase breast and ovarian cancer risk in patients [86]. Early studies demonstrated that tumour-associated mutations within the BRCA1 BRCT domains can disrupt its interaction with CtIP [2,6]. However, loss of BRCA1-CtIP interaction in mouse models expressing S327A mutant CtIP neither compromises genomic stability nor enhances tumour susceptibility [58,59]. Therefore, it remains an open question whether and how the BRCA1-CtIP interaction contributes to cancer development. Rebbeck et al. revealed that inherited clusters of SNPs at the *CtIP* locus ('CtIP haplotypes') are associated with modified breast, but not ovarian, cancer risk in women with *BRCA1* mutations, supporting a role for CtIP in tumour suppression [87]. Moreover, two different, deleterious *RBBP8* frameshift variants were recently detected in two early-onset breast cancer patients without *BRCA1* mutation, although their relative contribution to pathogenicity was not determined [88]. A recent study identified twelve rare germline *CtIP* mutations in Danish cohorts of early-onset breast cancer patients negative for high risk *BRCA1/2* mutations [78]. Remarkably, the CtIP-E804Δ variant, lacking a single amino acid within the C-terminal region (Fig. 1), caused severe defects in response to HU-induced replication stress, including deleterious nucleolytic degradation of reversed forks, but were proficient in DNA end resection and HR [78]. Owing to an exonic (A)9 mononucleotide repeat tract prone to mutation in mismatch repair-deficient tumours, *RBBP8* has been described as a prominent target for microsatellite instability (MSI)-induced mutations in colorectal, endometrial and myeloid malignancies [89–91].

Genomic alteration analyses in patient samples across 32 different cancer types reveal that *CtIP/RBBP8* is mainly amplified, but rarely mutated or deleted, which is in marked contrast to the situation of *BRCA1* (Fig. 3A). CtIP amplification is most prevalent in pancreatic cancers, with 16% of profiled tumours from two publicly available datasets showing genomic alterations in *RBBP8* (Fig. 3A and B). Although CtIP is predominantly amplified in pancreatic cancers, a few missense CtIP mutations of unknown significance have been reported that could potentially drive pancreatic tumorigenesis (Fig. 3B) [5]. Furthermore, Kaplan-Meier survival analysis in a study of 178 patients reveals that high expression of CtIP is significantly linked to poor prognosis in pancreatic adenocarcinoma (PAAD), suggesting an oncogenic activity of CtIP in these tumours (Fig. 3C).

Interestingly, *RBBP8* amplification was previously shown to occur mutually exclusive with *BRCA1* and *RB1* inactivation in high grade serous ovarian cancer, further supporting the idea of *RBBP8* acting as a candidate oncogene in certain tumour types [92]. Rb is a master negative regulator of cell cycle progression and numerous cancers are characterised by Rb inactivation [93]. It is therefore conceivable that CtIP upregulation could drive tumorigenesis in a similar manner to *RB1* loss-of-function. Presumably, CtIP-mediated Rb inhibition and subsequent de-repression of E2F-responsive genes such as *CCND1* could promote sustained proliferation by facilitating G1/S transition [39]. A similar relationship between CtIP and the Rb pathway has recently been proposed to contribute to the development of plasma cell myeloma, wherein high *RBBP8* expression correlates with poor survival and tumour recurrence in patients [94]. Accordingly, *CtIP* downregulation by promoter methylation was found to correlate with a more favourable prognosis in bladder cancer [95]. Yu et al. reported that CtIP expression was upregulated in gastric cancer and adjacent neoplastic tissues as compared with normal tissue [96]. CtIP overexpression in gastric cancer was accompanied by increased CtIP-CtBP-BRCA1-mediated *p21* suppression, which could promote G1/S transition and sustained proliferation [96]. Although CtIP expression levels did not significantly affect overall survival in those patients, CtIP-mediated proliferative activity, presumably through *p21* repression and *CCND1* upregulation (Fig. 2), was associated with worse prognosis at early stages of gastric cancer development [96]. Consistently, CtIP is frequently amplified in oesophageal carcinoma (ESCA) and stomach adenocarcinoma (STAD) (Fig. 3A). Other studies, however, have found that CtIP expression is frequently downregulated in breast cancer patients and that low expression is combined with unfavourable prognosis and metastasis formation [97–99], which is markedly different from what is observed in pancreatic cancer patients (Fig. 3C). Thus, the relationship between CtIP expression and disease progression appears to be intricately linked with

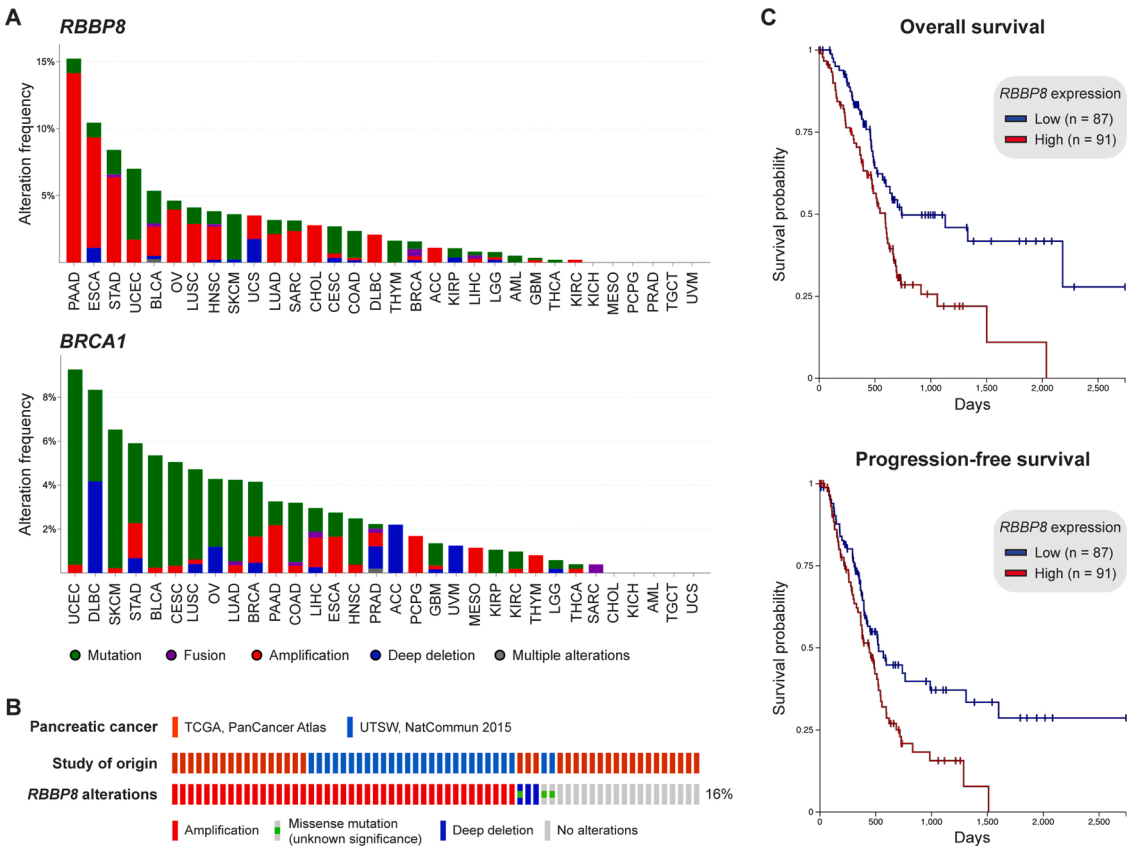

Fig. 3. *RBBP8* genomic alterations and expression analysis in cancer.
(A) Cross-cancer summary of mutations and copy number variations of *RBBP8* and *BRCA1* across 32 cancer types based on patient data from the combined TCGA PanCancer Atlas [126] available on cBioPortal (http://www.cbioportal.org/). **(B)** *RBBP8* amplification is the most prevalent type of genomic alteration observed in two independent pancreatic cancer studies (TCGA, PanCancer Atlas for pancreatic adenocarcinoma (PAAD); UTSW, NatComm, 2015) [126,127]. **(C)** Kaplan-Meier plots using mRNA expression data from primary tumour samples of the TCGA Pan-Cancer Atlas PAAD study [126] were generated in UCSC Xena [128]. High CtIP expression correlates with unfavourable prognosis in PAAD, both for overall (p-value = 0.0032) and progression-free (p-value = 0.0092) survival. For this analysis, patient samples were divided into two *RBBP8* expression groups, separated by the median: "High" (expression values $\geq$ 9.350) and "Low" (expression values $<$ 9.350).

tumour type, possibly reflecting the relative importance of distinct CtIP functions in the pathogenesis of different cancers.

3.2. Anti- and pro-tumorigenic properties of CtIP

Owing to its fundamental role in HR, CtIP may, albeit to a limited extent, be involved in tumour suppression through preventing the accumulation of cancer-enabling mutations and genome instability arising from erroneous repair of DSBs [1,2,82]. Originally, CtIP has been postulated as a haploinsufficient tumour suppressor based on the shortened lifespan of *Ctip*$^{+/-}$ heterozygous mice, which succumbed to multiple tumours, predominantly large B-cell lymphomas [45]. However, a recent independent study could not recapitulate this finding, showing instead that tumour-free survival is indistinguishable between wild-type mice and mice harbouring a single null allele of the *Ctip* gene [100]. Moreover, and considerably different from conditional *Brca1*-null females, mammary-specific biallelic inactivation of *Ctip* did not induce breast tumours [100]. On the contrary, Reczek et al. discovered that *Ctip* loss-of-function slowed down mammary tumorigenesis in p53-deficient mice, suggesting an oncogenic activity in CtIP [100]. A conceivable mechanistic explanation for CtIP-driven malignant transformation is that CtIP-dependent resection promotes chromosomal translocations and telomere fusions through a-EJ (Fig. 2) [101–103]. In line with this hypothesis, Srinivasan et al. recently reported that miR223-3p-mediated CtIP downregulation prevents mutagenic a-EJ and, thus, chromosomal translocations in normal cells, whilst reduced expression of miR223-3p is frequently observed in BRCA1-deficient cancer cells [44].

Maintenance of telomere length and integrity is essential for preventing genomic instability and oncogenic transformation [104]. Due to the formation of secondary structures and the repetitive nature of telomeric DNA sequences, telomere replication is a complex process that can lead to replication fork stalling and collapse [105]. CtIP was recently shown to protect replicating telomeres from aberrant shortening and fusions, by rescuing stalled replication forks and promoting HR-mediated DSB repair of collapsed forks [74]. In addition, the function of CtIP in protecting nascent DNA at stalled forks from nucleolytic degradation (Fig. 2) may contribute to the protection of replicating telomeres [77]. Furthermore, work from the Scully laboratory revealed that CtIP acts independently from BRCA1 in preventing the formation of microhomology-mediated small tandem duplications, a genome-wide DNA rearrangement signature produced by aberrant processing of stalled replication forks and typically found in BRCA1-deficient cancers [106,107]. Therefore, it is tempting to speculate that CtIP, at least in parts, counteracts tumorigenesis via its role in alleviating replication stress-induced genomic instability.

4. Clinical relevance of targeting CtIP for cancer therapy

Despite its ambiguous role in tumorigenesis, CtIP has prognostic value in several types of cancer and could serve as a predictive biomarker for treatment response in selected patients [94,95,98,99]. As previously mentioned, low *RBBP8* expression correlates with breast

cancer aggressiveness and poor response to hormonal therapy in breast cancer [97,99]. However, reduced CtIP protein levels in luminal breast cancers are associated with better response to DNA-damaging chemotherapy [99], presumably due to a decreased DSB repair capacity. In bladder cancer, CtIP promoter methylation, leading to gene silencing, is linked to better prognosis and can be readily detected in non-invasive urine tests [95]. Platinum-based drugs are commonly used for treating bladder cancer [108] and have demonstrated improved efficacy in *BRCA1/2*-mutated, HR-deficient tumours [109,110]. Therefore, it is plausible that reduced CtIP expression and ensuing HR defects could underlie improved response to cisplatin treatment in bladder cancer. In contrast, selective CtIP inhibition with yet-to-be-discovered targeting molecules may synergise with DNA-damaging drugs, particularly in tumours displaying CtIP amplification such as highly aggressive pancreatic cancer (Fig. 3).

Genomic instability and acquired DNA repair defects are common features of many cancers and can be therapeutically exploited based on the concept of synthetic lethality [82,110–113]. A paradigm example is the use of PARP inhibitors for treating *BRCA1/2*-mutated breast and ovarian cancer [114–116]. A major caveat of PARPi therapy is the development of treatment resistance due to secondary mutations leading to the restoration of BRCA1/2 or re-establishment of DNA end resection [117,118]. Therefore, selectively targeting additional DNA damage response components, including CtIP, could potentially re-sensitise these cancers to PARPi [119]. CtIP depletion sensitises breast cancer cells to PARPi, presumably by suppressing HR, suggesting that CtIP could be an interesting biomarker for predicting disease progression and therapy response in breast cancer [98]. Furthermore, impaired recruitment of CtIP to DSBs upon CHD1 loss was shown to sensitise prostate cancer cells to PARPi [120]. Ceccaldi et al. reported that HR-deficient ovarian cancers strongly rely on Polθ-mediated a-EJ to repair DSBs arising at collapsed forks, thereby contributing to tumour development [112,121,122]. Thus, blocking a-EJ via Polθ or CtIP inhibition could be a valid synthetic lethal strategy for selective killing of BRCA1-deficient tumours. We have recently reported that CtIP and BRCA1 operate in separate fork protection pathways, thus providing an alternative mechanism explaining the potential synthetic lethal relationship between CtIP and BRCA1 [77]. A recent synthetic lethality screen for candidate PARPi sensitivity genes in HR-proficient cells identified BRD4, a chromatin-binding protein frequently overexpressed in several types of cancers [123,124]. Remarkably, BRD4 enrichment at the *RBBP8* promoter and enhancer, and, thus, CtIP expression, were significantly decreased upon BRD4 inhibition, which ultimately caused HR deficiency and PARPi hypersensitivity [123]. In our opinion, these findings provide a clear rationale for the development of CtIP targeting approaches to potentially improve existing cancer therapies and counteract acquired PARPi resistance in selected cancers.

5. Conclusions

CtIP is a multifaceted player in DNA repair and genomic stability and acts as a “double-edged sword” in cancer. Its diverse functions in transcriptional regulation, DSB repair and replication stress response have been implicated in different aspects of tumorigenesis, leading to positive or unfavourable outcomes depending on cancer type and biological context. Thus, CtIP (*RBBP8*) can be classified as ‘double agent’, a term coined for genes harbouring both tumour suppressive and oncogenic functions, depending on the respective alterations and cellular context, and often functioning as central hubs in protein interaction networks [125]. Further research will be required to more clearly define the role of CtIP in maintaining the balance between oncogenesis and tumour suppression, with potential implications for cancer prognosis and therapy.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

Work in the Sartori laboratory is supported by the Swiss National Science Foundation (grant number: 31003A_176161) and the Swiss Cancer Research Foundation (grant number: KFS-4702-02-2019). We would like to thank Anika Trenner for critically reading the manuscript and apologise to any authors whose work could not be cited due to space limitations.

References

[1] S.N. Andres, R.S. Williams, CtIP/Ctp1/Sae2, molecular form fit for function, DNA Repair. 56 (2017) 109–117, https://doi.org/10.1016/j.dnarep.2017.06.013.
[2] N. Makharashvili, T.T. Paull, CtIP: A DNA damage response protein at the intersection of DNA metabolism, DNA Repair. 32 (2015) 75–81, https://doi.org/10.1016/j.dnarep.2015.04.016.
[3] U. Schaeper, T. Subramanian, L. Lim, J.M. Boyd, G. Chinnadurai, Interaction between a Cellular Protein That Binds to the C-terminal Region of Adenovirus E1A (CtBP) and a Novel Cellular Protein Is Disrupted by E1A through a Conserved PLDLS Motif, J. Biol. Chem. 273 (1998) 8549–8552, https://doi.org/10.1074/jbc.273.15.8549.
[4] C. Fusco, A. Reymond, A.S. Zervos, Molecular Cloning and Characterization of a Novel Retinoblastoma-Binding Protein, Genomics. 51 (1998) 351–358, https://doi.org/10.1006/geno.1998.5368.
[5] A.K. Wong, P.A. Ormonde, R. Pero, Y. Chen, L. Lian, G. Salada, S. Berry, Q. Lawrence, P. Dayananth, P. Ha, S.V. Tavtigian, D.H.-F. Teng, P.L. Bartel, Characterization of a carboxy-terminal BRCA1 interacting protein, Oncogene. 17 (1998) 2279–2285, https://doi.org/10.1038/sj.onc.1202150.
[6] X. Yu, L.C. Wu, A.M. Bowcock, A. Aronheim, R. Baer, The C-terminal (BRCT) Domains of BRCA1 Interact in Vivo with CtIP, a Protein Implicated in the CtBP Pathway of Transcriptional Repression, J. Biol. Chem. 273 (1998) 25388–25392, https://doi.org/10.1074/jbc.273.39.25388.
[7] G. Chinnadurai, CtIP, a candidate tumor susceptibility gene is a team player with luminaries, Biochimica et Biophysica Acta (BBA) - Reviews on Cancer 1765 (2006) 67–73, https://doi.org/10.1016/j.bbcan.2005.09.002.
[8] Z. You, J.M. Bailis, DNA damage and decisions: CtIP coordinates DNA repair and cell cycle checkpoints, Trends in Cell Biology. 20 (2010) 402–409, https://doi.org/10.1016/j.tcb.2010.04.002.
[9] J. Godau, L.P. Ferretti, A. Trenner, E. Dubois, C. von Aesch, A. Marmignon, L. Simon, A. Kapusta, R. Guérois, M. Bétermier, A.A. Sartori, Identification of a miniature Sae2/Ctp1/CtIP ortholog from Paramecium tetraurelia required for sexual reproduction and DNA double-strand break repair, DNA Repair. 77 (2019) 96–108, https://doi.org/10.1016/j.dnarep.2019.03.011.
[10] S. Prinz, A. Amon, F. Klein, Isolation of COM1, a New Gene Required to Complete Meiotic Double-Strand Break-Induced Recombination in Saccharomyces cerevisiae, Genetics. 146 (1997) 781–795.
[11] A.H.Z. McKee, N. Kleckner, A General Method for Identifying Recessive Diploid-Specific Mutations in Saccharomyces cerevisiae, Its Application to the Isolation of Mutants Blocked at Intermediate Stages of Meiotic Prophase and Characterization of a New Gene SAE2, Genetics. 146 (1997) 797–816.
[12] A.A. Sartori, C. Lukas, J. Coates, M. Mistrik, S. Fu, J. Bartek, R. Baer, J. Lukas, S. P. Jackson, Human CtIP promotes DNA end resection, Nature. 450 (2007) 509–514, https://doi.org/10.1038/nature06337.
[13] L.S. Symington, Mechanism and regulation of DNA end resection in eukaryotes, Critical Reviews in Biochemistry and Molecular Biology. 51 (2016) 195–212, https://doi.org/10.3109/10409238.2016.1172552.
[14] R. Scully, A. Panday, R. Elango, N.A. Willis, DNA double-strand break repair-pathway choice in somatic mammalian cells, Nat Rev Mol Cell Biol. (2019), https://doi.org/10.1038/s41580-019-0152-0.
[15] M.J. Dubin, P.H. Stokes, E.Y.M. Sum, R.S. Williams, V.A. Valova, P.J. Robinson, G.J. Lindeman, J.N.M. Glover, J.E. Visvader, J.M. Matthews, Dimerization of CtIP, a BRCA1- and CtBP-interacting Protein, Is Mediated by an N-terminal Coiled-coil Motif, J. Biol. Chem. 279 (2004) 26932–26938, https://doi.org/10.1074/jbc.M313974200.
[16] H. Wang, Z. Shao, L.Z. Shi, P.Y.-H. Hwang, L.N. Truong, M.W. Berns, D.J. Chen, X. Wu, CtIP Protein Dimerization Is Critical for Its Recruitment to Chromosomal DNA Double-stranded Breaks, J. Biol. Chem. 287 (2012) 21471–21480, https://doi.org/10.1074/jbc.M112.355354.
[17] O.R. Davies, J.V. Forment, M. Sun, R. Belotserkovskaya, J. Coates, Y. Galanty, M. Demir, C.R. Morton, N.J. Rzechorzek, S.P. Jackson, L. Pellegrini, CtIP tetramer assembly is required for DNA-end resection and repair, Nat Struct Mol Biol. 22 (2015) 150–157, https://doi.org/10.1038/nsmb.2937.
[18] O.J. Wilkinson, A. Martín-González, H. Kang, S.J. Northall, D.B. Wigley, F. Moreno-Herrero, M.S. Dillingham, CtIP forms a tetrameric dumbbell-shaped particle which bridges complex DNA end structures for double-strand break repair, ELife. 8 (2019), e42129, https://doi.org/10.7554/eLife.42129.
[19] S.N. Andres, C.D. Appel, J.W. Westmoreland, J.S. Williams, Y. Nguyen, P. D. Robertson, M.A. Resnick, R.S. Williams, Tetrameric Ctp1 coordinates DNA

binding and DNA bridging in DNA double-strand-break repair, Nat Struct Mol Biol. 22 (2015) 158–166, https://doi.org/10.1038/nsmb.2945.
[20] S.N. Andres, Z.M. Li, D.A. Erie, R.S. Williams, Ctp1 protein–DNA filaments promote DNA bridging and DNA double-strand break repair, J. Biol. Chem. 294 (2019) 3312–3320, https://doi.org/10.1074/jbc.RA118.006759.
[21] N. Makharashvili, A.T. Tubbs, S.-H. Yang, H. Wang, O. Barton, Y. Zhou, R. A. Deshpande, J.-H. Lee, M. Lobrich, B.P. Sleckman, X. Wu, T.T. Paull, Catalytic and Noncatalytic Roles of the CtIP Endonuclease in Double-Strand Break End Resection, Molecular Cell. 54 (2014) 1022–1033, https://doi.org/10.1016/j.molcel.2014.04.011.
[22] H. Wang, Y. Li, L.N. Truong, L.Z. Shi, P.Y.-H. Hwang, J. He, J. Do, M.J. Cho, H. Li, A. Negrete, J. Shiloach, M.W. Berns, B. Shen, L. Chen, X. Wu, CtIP Maintains Stability at Common Fragile Sites and Inverted Repeats by End Resection-Independent Endonuclease Activity, Molecular Cell. 54 (2014) 1012–1021, https://doi.org/10.1016/j.molcel.2014.04.012.
[23] R. Anand, L. Ranjha, E. Cannavo, P. Cejka, Phosphorylated CtIP Functions as a Co-factor of the MRE11-RAD50-NBS1 Endonuclease in DNA End Resection, Molecular Cell. 64 (2016) 940–950, https://doi.org/10.1016/j.molcel.2016.10.017.
[24] L.P. Ferretti, L. Lafranchi, A.A. Sartori, Controlling DNA-end resection: a new task for CDKs, Front. Genet. 4 (2013), https://doi.org/10.3389/fgene.2013.00099.
[25] X. Yu, J. Chen, DNA Damage-Induced Cell Cycle Checkpoint Control Requires CtIP, a Phosphorylation-Dependent Binding Partner of BRCA1 C-Terminal Domains, Molecular and Cellular Biology. 24 (2004) 9478–9486, https://doi.org/10.1128/MCB.24.21.9478-9486.2004.
[26] A. Cruz-García, A. López-Saavedra, P. Huertas, BRCA1 Accelerates CtIP-Mediated DNA-End Resection, Cell Reports. 9 (2014) 451–459, https://doi.org/10.1016/j.celrep.2014.08.076.
[27] P. Huertas, S.P. Jackson, Human CtIP Mediates Cell Cycle Control of DNA End Resection and Double Strand Break Repair, J. Biol. Chem. 284 (2009) 9558–9565, https://doi.org/10.1074/jbc.M808906200.
[28] M. Steger, O. Murina, D. Hühn, L.P. Ferretti, R. Walser, K. Hänggi, L. Lafranchi, C. Neugebauer, S. Paliwal, P. Janscak, B. Gerrits, G. Del Sal, O. Zerbe, A. A. Sartori, Prolyl Isomerase PIN1 Regulates DNA Double-Strand Break Repair by Counteracting DNA End Resection, Molecular Cell. 50 (2013) 333–343, https://doi.org/10.1016/j.molcel.2013.03.023.
[29] X.Z. Zhou, K.P. Lu, The isomerase PIN1 controls numerous cancer-driving pathways and is a unique drug target, Nat Rev Cancer. 16 (2016) 463–478, https://doi.org/10.1038/nrc.2016.49.
[30] W. Pu, Y. Zheng, Y. Peng, Prolyl Isomerase Pin1 in Human Cancer: Function, Mechanism, and Significance, Front. Cell Dev. Biol. 8 (2020), https://doi.org/10.3389/fcell.2020.00168.
[31] L.P. Ferretti, S.-F. Himmels, A. Trenner, C. Walker, C. von Aesch, A. Eggenschwiler, O. Murina, R.I. Enchev, M. Peter, R. Freire, A. Porro, A. A. Sartori, Cullin3-KLHL15 ubiquitin ligase mediates CtIP protein turnover to fine-tune DNA-end resection, Nat Commun. 7 (2016) 1–16, https://doi.org/10.1038/ncomms12628.
[32] L. Lafranchi, H.R. de Boer, E.G. de Vries, S.-E. Ong, A.A. Sartori, M.A. van Vugt, APC/CCdh1 controls CtIP stability during the cell cycle and in response to DNA damage, The EMBO Journal 33 (2014) 2860–2879, https://doi.org/10.15252/embj.201489017.
[33] S.E. Peterson, Y. Li, F. Wu-Baer, B.T. Chait, R. Baer, H. Yan, M.E. Gottesman, J. Gautier, Activation of DSB Processing Requires Phosphorylation of CtIP by ATR, Molecular Cell. 49 (2013) 657–667, https://doi.org/10.1016/j.molcel.2012.11.020.
[34] H. Wang, L.Z. Shi, C.C.L. Wong, X. Han, P.Y.-H. Hwang, L.N. Truong, Q. Zhu, Z. Shao, D.J. Chen, M.W. Berns, J.R. Yates, L. Chen, X. Wu, The Interaction of CtIP and Nbs1 Connects CDK and ATM to Regulate HR–Mediated Double-Strand Break Repair, PLoS Genet. 9 (2013), e1003277, https://doi.org/10.1371/journal.pgen.1003277.
[35] R.A. Deshpande, L.R. Myler, M.M. Soniat, N. Makharashvili, L. Lee, S.P. Lees-Miller, I.J. Finkelstein, T.T. Paull, DNA-dependent protein kinase promotes DNA end processing by MRN and CtIP, Science Advances. 6 (2020), eaay0922, https://doi.org/10.1126/sciadv.aay0922.
[36] O. Murina, C. von Aesch, U. Karakus, L.P. Ferretti, H.A. Bolck, K. Hänggi, A. A. Sartori, FANCD2 and CtIP Cooperate to Repair DNA Interstrand Crosslinks, Cell Reports. 7 (2014) 1030–1038, https://doi.org/10.1016/j.celrep.2014.03.069.
[37] J. Unno, A. Itaya, M. Taoka, K. Sato, J. Tomida, W. Sakai, K. Sugasawa, M. Ishiai, T. Ikura, T. Isobe, H. Kurumizaka, M. Takata, FANCD2 Binds CtIP and Regulates DNA-End Resection during DNA Interstrand Crosslink Repair, Cell Reports. 7 (2014) 1039–1047, https://doi.org/10.1016/j.celrep.2014.04.005.
[38] P.H. Stokes, C.W. Liew, A.H. Kwan, P. Foo, H.E. Barker, A. Djamirze, V. O'Reilly, J.E. Visvader, J.P. Mackay, J.M. Matthews, Structural Basis of the Interaction of the Breast Cancer Oncogene LMO4 with the Tumour Suppressor CtIP/RBBP8, Journal of Molecular Biology. 425 (2013) 1101–1110, https://doi.org/10.1016/j.jmb.2013.01.017.
[39] F. Liu, W.-H. Lee, CtIP Activates Its Own and Cyclin D1 Promoters via the E2F/RB Pathway during G1/S Progression, Molecular and Cellular Biology. 26 (2006) 3124–3134, https://doi.org/10.1128/MCB.26.8.3124-3134.2006.
[40] N.T. Martin, K. Nakamura, R. Davies, S.A. Nahas, C. Brown, R. Tunuguntla, R. A. Gatti, H. Hu, ATM–Dependent MiR-335 Targets CtIP and Modulates the DNA Damage Response, PLOS Genetics. 9 (2013), e1003505, https://doi.org/10.1371/journal.pgen.1003505.
[41] A.-H. Rezaeian, H. Khanbabaei, G.A. Calin, Therapeutic Potential of the miRNA–ATM Axis in the Management of Tumor Radioresistance, Cancer Res. 80 (2020) 139–150, https://doi.org/10.1158/0008-5472.CAN-19-1807.
[42] D. Hühn, A.N. Kousholt, C.S. Sørensen, A.A. Sartori, miR-19, a component of the oncogenic miR-17~92 cluster, targets the DNA-end resection factor CtIP, Oncogene. 34 (2015) 3977–3984, https://doi.org/10.1038/onc.2014.329.
[43] L.-L. Fang, X.-H. Wang, B.-F. Sun, X.-D. Zhang, X.-H. Zhu, Z.-J. Yu, H. Luo, Expression, regulation and mechanism of action of the miR-17-92 cluster in tumor cells (Review), International Journal of Molecular Medicine. 40 (2017) 1624–1630, https://doi.org/10.3892/ijmm.2017.3164.
[44] G. Srinivasan, E.A. Williamson, K. Kong, A.S. Jaiswal, G. Huang, H.-S. Kim, O. Schärer, W. Zhao, S. Burma, P. Sung, R. Hromas, MiR223-3p promotes synthetic lethality in BRCA1-deficient cancers, PNAS. 116 (2019) 17438–17443, https://doi.org/10.1073/pnas.1903150116.
[45] P.-L. Chen, F. Liu, S. Cai, X. Lin, A. Li, Y. Chen, B. Gu, E.Y.-H.P. Lee, W.-H. Lee, Inactivation of CtIP Leads to Early Embryonic Lethality Mediated by G1 Restraint and to Tumorigenesis by Haploid Insufficiency, Molecular and Cellular Biology. 25 (2005) 3535–3542, https://doi.org/10.1128/MCB.25.9.3535-3542.2005.
[46] B. Liu, R. Cong, B. Peng, B. Zhu, G. Dou, H. Ai, X. Zhang, Z. Wang, X. Xu, CtIP is required for DNA damage-dependent induction of P21, Cell Cycle. 13 (2014) 90–95, https://doi.org/10.4161/cc.26810.
[47] S. Li, P.-L. Chen, T. Subramanian, G. Chinnadurai, G. Tomlinson, C.K. Osborne, Z. D. Sharp, W.-H. Lee, Binding of CtIP to the BRCT Repeats of BRCA1 Involved in the Transcription Regulation of p21 Is Disrupted Upon DNA Damage, J. Biol. Chem. 274 (1999) 11334–11338, https://doi.org/10.1074/jbc.274.16.11334.
[48] S. Li, N.S.Y. Ting, L. Zheng, P.-L. Chen, Y. Ziv, Y. Shiloh, E.Y.-H.P. Lee, W.-H. Lee, Functional link of BRCA1 and ataxia telangiectasia gene product in DNA damage response, Nature. 406 (2000) 210–215, https://doi.org/10.1038/35018134.
[49] S. Furuta, J.-M. Wang, S. Wei, Y.-M. Jeng, X. Jiang, B. Gu, P.-L. Chen, E.Y.-H. P. Lee, W.-H. Lee, Removal of BRCA1/CtIP/ZBRK1 repressor complex on ANG1 promoter leads to accelerated mammary tumor growth contributed by prominent vasculature, Cancer Cell. 10 (2006) 13–24, https://doi.org/10.1016/j.ccr.2006.05.022.
[50] K.M. Ahmed, C.Y. Tsai, W.-H. Lee, Derepression of HMGA2 via Removal of ZBRK1/BRCA1/CtIP Complex Enhances Mammary Tumorigenesis, J. Biol. Chem. 285 (2010) 4464–4471, https://doi.org/10.1074/jbc.M109.062265.
[51] E.Y.M. Sum, B. Peng, X. Yu, J. Chen, J. Byrne, G.J. Lindeman, J.E. Visvader, The LIM Domain Protein LMO4 Interacts with the Cofactor CtIP and the Tumor Suppressor BRCA1 and Inhibits BRCA1 Activity, J. Biol. Chem. 277 (2002) 7849–7856, https://doi.org/10.1074/jbc.M110603200.
[52] H. Chen, J. Shan, D. Chen, R. Wang, W. Qi, H. Wang, Y. Ke, W. Liu, X. Zeng, CtIP promotes G2/M arrest in etoposide-treated HCT116 cells in a p53-independent manner, Journal of Cellular Physiology. 234 (2019) 11871–11881, https://doi.org/10.1002/jcp.27824.
[53] A. Jazayeri, J. Falck, C. Lukas, J. Bartek, G.C.M. Smith, J. Lukas, S.P. Jackson, ATM- and cell cycle-dependent regulation of ATR in response to DNA double-strand breaks, Nat Cell Biol. 8 (2006) 37–45, https://doi.org/10.1038/ncb1337.
[54] Z. You, L.Z. Shi, Q. Zhu, P. Wu, Y.-W. Zhang, A. Basilio, N. Tonnu, I.M. Verma, M. W. Berns, T. Hunter, CtIP Links DNA Double-Strand Break Sensing to Resection, Molecular Cell. 36 (2009) 954–969, https://doi.org/10.1016/j.molcel.2009.12.002.
[55] F. Marini, C.C. Rawal, G. Liberi, A. Pellicioli, Regulation of DNA Double Strand Breaks Processing: Focus on Barriers, Front. Mol. Biosci. 6 (2019), https://doi.org/10.3389/fmolb.2019.00055.
[56] M. Tarsounas, P. Sung, The antitumorigenic roles of BRCA1–BARD1 in DNA repair and replication, Nat Rev Mol Cell Biol. (2020) 1–16, https://doi.org/10.1038/s41580-020-0218-z.
[57] K. Karanam, R. Kafri, A. Loewer, G. Lahav, Quantitative Live Cell Imaging Reveals a Gradual Shift between DNA Repair Mechanisms and a Maximal Use of HR in Mid S Phase, Molecular Cell. 47 (2012) 320–329, https://doi.org/10.1016/j.molcel.2012.05.052.
[58] F. Polato, E. Callen, N. Wong, R. Faryabi, S. Bunting, H.-T. Chen, M. Kozak, M. J. Kruhlak, C.R. Reczek, W.-H. Lee, T. Ludwig, R. Baer, L. Feigenbaum, S. Jackson, A. Nussenzweig, CtIP-mediated resection is essential for viability and can operate independently of BRCA1, Journal of Experimental Medicine. 211 (2014) 1027–1036, https://doi.org/10.1084/jem.20131939.
[59] C.R. Reczek, M. Szabolcs, J.M. Stark, T. Ludwig, R. Baer, The interaction between CtIP and BRCA1 is not essential for resection-mediated DNA repair or tumor suppression, J Cell Biol. 201 (2013) 693–707, https://doi.org/10.1083/jcb.201302145.
[60] W. Eid, M. Steger, M. El-Shemerly, L.P. Ferretti, J. Peña-Diaz, C. König, E. Valtorta, A.A. Sartori, S. Ferrari, DNA end resection by CtIP and exonuclease 1 prevents genomic instability, EMBO Reports. 11 (2010) 962–968, https://doi.org/10.1038/embor.2010.157.
[61] J.M. Daley, J. Jimenez-Sainz, W. Wang, A.S. Miller, X. Xue, K.A. Nguyen, R. B. Jensen, P. Sung, Enhancement of BLM-DNA2-Mediated Long-Range DNA End Resection by CtIP, Cell Reports. 21 (2017) 324–332, https://doi.org/10.1016/j.celrep.2017.09.048.
[62] I. Ceppi, S.M. Howard, K. Kasaciunaite, C. Pinto, R. Anand, R. Seidel, P. Cejka, CtIP promotes the motor activity of DNA2 to accelerate long-range DNA end resection, PNAS. (2020), https://doi.org/10.1073/pnas.2001165117.
[63] A. Maréchal, L. Zou, RPA-coated single-stranded DNA as a platform for post-translational modifications in the DNA damage response, Cell Res. 25 (2015) 9–23, https://doi.org/10.1038/cr.2014.147.
[64] H.H.Y. Chang, N.R. Pannunzio, N. Adachi, M.R. Lieber, Non-homologous DNA end joining and alternative pathways to double-strand break repair, Nat Rev Mol Cell Biol. 18 (2017) 495–506, https://doi.org/10.1038/nrm.2017.48.
[65] P.A. Mateos-Gomez, T. Kent, S.K. Deng, S. McDevitt, E. Kashkina, T.M. Hoang, R. T. Pomerantz, A. Sfeir, The helicase domain of Polθ counteracts RPA to promote

alt-NHEJ, Nat Struct Mol Biol. 24 (2017) 1116–1123, https://doi.org/10.1038/nsmb.3494.
[66] O. Barton, S.C. Naumann, R. Diemer-Biehs, J. Künzel, M. Steinlage, S. Conrad, N. Makharashvili, J. Wang, L. Feng, B.S. Lopez, T.T. Paull, J. Chen, P.A. Jeggo, M. Löbrich, Polo-like kinase 3 regulates CtIP during DNA double-strand break repair in G1, J Cell Biol. 206 (2014) 877–894, https://doi.org/10.1083/jcb.201401146.
[67] R. Biehs, M. Steinlage, O. Barton, S. Juhász, J. Künzel, J. Spies, A. Shibata, P. A. Jeggo, M. Löbrich, DNA Double-Strand Break Resection Occurs during Non-homologous End Joining in G1 but Is Distinct from Resection during Homologous Recombination, Molecular Cell. 65 (2017) 671–684, https://doi.org/10.1016/j.molcel.2016.12.016, e5.
[68] M.K. Zeman, K.A. Cimprich, Causes and consequences of replication stress, Nat Cell Biol. 16 (2014) 2–9, https://doi.org/10.1038/ncb2897.
[69] H. Gaillard, T. García-Muse, A. Aguilera, Replication stress and cancer, Nat Rev Cancer. 15 (2015) 276–289, https://doi.org/10.1038/nrc3916.
[70] H. Dungrawala, K.L. Rose, K.P. Bhat, K.N. Mohni, G.G. Glick, F.B. Couch, D. Cortez, The Replication Checkpoint Prevents Two Types of Fork Collapse without Regulating Replisome Stability, Molecular Cell. 59 (2015) 998–1010, https://doi.org/10.1016/j.molcel.2015.07.030.
[71] S.R. Wessel, K.N. Mohni, J.W. Luzwick, H. Dungrawala, D. Cortez, Functional Analysis of the Replication Fork Proteome Identifies BET Proteins as PCNA Regulators, Cell Reports. 28 (2019) 3497–3509, https://doi.org/10.1016/j.celrep.2019.08.051, e4.
[72] B. Gu, P.-L. Chen, Expression of PCNA-binding domain of CtIP, a motif required for CtIP localization at DNA replication foci, causes DNA damage and activation of DNA damage checkpoint, Cell Cycle. 8 (2009) 1409–1420, https://doi.org/10.4161/cc.8.9.8322.
[73] J.E. Yeo, E.H. Lee, E.A. Hendrickson, A. Sobeck, CtIP mediates replication fork recovery in a FANCD2-regulated manner, Hum Mol Genet. 23 (2014) 3695–3705, https://doi.org/10.1093/hmg/ddu078.
[74] S. Stroik, K. Kurtz, E.A. Hendrickson, CtIP is essential for telomere replication, Nucleic Acids Research (2019), https://doi.org/10.1093/nar/gkz652 gkz652.
[75] D. Lemaçon, J. Jackson, A. Quinet, J.R. Brickner, S. Li, S. Yazinski, Z. You, G. Ira, L. Zou, N. Mosammaparast, A. Vindigni, MRE11 and EXO1 nucleases degrade reversed forks and elicit MUS81-dependent fork rescue in BRCA2-deficient cells, Nat Commun. 8 (2017) 860, https://doi.org/10.1038/s41467-017-01180-5.
[76] F. Coquel, M.-J. Silva, H. Técher, K. Zadorozhny, S. Sharma, J. Nieminuszczy, C. Mettling, E. Dardillac, A. Barthe, A.-L. Schmitz, A. Promonet, A. Cribier, A. Sarrazin, W. Niedzwiedz, B. Lopez, V. Costanzo, L. Krejci, A. Chabes, M. Benkirane, Y.-L. Lin, P. Pasero, SAMHD1 acts at stalled replication forks to prevent interferon induction, Nature. 557 (2018) 57–61, https://doi.org/10.1038/s41586-018-0050-1.
[77] S. Przetocka, A. Porro, H.A. Bolck, C. Walker, A. Lezaja, A. Trenner, C. von Aesch, S.-F. Himmels, A.D. D'Andrea, R. Ceccaldi, M. Altmeyer, A.A. Sartori, CtIP-Mediated Fork Protection Synergizes with BRCA1 to Suppress Genomic Instability upon DNA Replication Stress, Molecular Cell. 72 (2018) 568–582, https://doi.org/10.1016/j.molcel.2018.09.014, e6.
[78] R. Zarrizi, M.R. Higgs, K. Voßgröne, M. Rossing, B. Bertelsen, M. Bose, A. N. Kousholt, H. Rösner, the C. Network, B. Ejlertsen, G.S. Stewart, F.C. Nielsen, C. S. Sørensen, Germline RBBP8 variants associated with early-onset breast cancer compromise replication fork stability, Journal of Clinical Investigation. (2020), https://doi.org/10.1172/JCI127521.
[79] T. Yasuhara, R. Kato, Y. Hagiwara, B. Shiotani, M. Yamauchi, S. Nakada, A. Shibata, K. Miyagawa, Human Rad52 Promotes XPG-Mediated R-loop Processing to Initiate Transcription-Associated Homologous Recombination Repair, Cell. 175 (2018) 558–570, https://doi.org/10.1016/j.cell.2018.08.056, e11.
[80] N. Makharashvili, S. Arora, Y. Yin, Q. Fu, X. Wen, J.-H. Lee, C.-H. Kao, J. W. Leung, K.M. Miller, T.T. Paull, Sae2/CtIP prevents R-loop accumulation in eukaryotic cells, ELife. 7 (2018), e42733, https://doi.org/10.7554/eLife.42733.
[81] M. Daza-Martin, K. Starowicz, M. Jamshad, S. Tye, G.E. Ronson, H.L. MacKay, A. S. Chauhan, A.K. Walker, H.R. Stone, J.F.J. Beesley, J.L. Coles, A.J. Garvin, G. S. Stewart, T.J. McCorvie, X. Zhang, R.M. Densham, J.R. Morris, Isomerization of BRCA1–BARD1 promotes replication fork protection, Nature. 571 (2019) 521–527, https://doi.org/10.1038/s41586-019-1363-4.
[82] D. Hanahan, R.A. Weinberg, Hallmarks of Cancer: The Next Generation, Cell. 144 (2011) 646–674, https://doi.org/10.1016/j.cell.2011.02.013.
[83] P. Qvist, P. Huertas, S. Jimeno, M. Nyegaard, M.J. Hassan, S.P. Jackson, A. D. Børglum, CtIP Mutations Cause Seckel and Jawad Syndromes, PLoS Genet. 7 (2011), e1002310, https://doi.org/10.1371/journal.pgen.1002310.
[84] G.K. Alderton, H. Joenje, R. Varon, A.D. Børglum, P.A. Jeggo, M. O'Driscoll, Seckel syndrome exhibits cellular features demonstrating defects in the ATR-signalling pathway, Hum Mol Genet. 13 (2004) 3127–3138, https://doi.org/10.1093/hmg/ddh335.
[85] A.N. Kousholt, K. Fugger, S. Hoffmann, B.D. Larsen, T. Menzel, A.A. Sartori, C. S. Sørensen, CtIP-dependent DNA resection is required for DNA damage checkpoint maintenance but not initiation, J Cell Biol. 197 (2012) 869–876, https://doi.org/10.1083/jcb.201111065.
[86] C.-C. Chen, W. Feng, P.X. Lim, E.M. Kass, M. Jasin, Homology-Directed Repair and the Role of BRCA1, BRCA2, and Related Proteins in Genome Integrity and Cancer, Annu. Rev. Cancer Biol. 2 (2018) 313–336, https://doi.org/10.1146/annurev-cancerbio-030617-050502.
[87] T.R. Rebbeck, N. Mitra, S.M. Domchek, F. Wan, T.M. Friebel, T.V. Tran, C. F. Singer, M.-K.M. Tea, J.L. Blum, N. Tung, O.I. Olopade, J.N. Weitzel, H. T. Lynch, C.L. Snyder, J.E. Garber, A.C. Antoniou, S. Peock, D.G. Evans, J. Paterson, M.J. Kennedy, A. Donaldson, H. Dorkins, D.F. Easton, W. S. Rubinstein, M.B. Daly, C. Isaacs, H. Nevanlinna, F.J. Couch, I.L. Andrulis, E. Freidman, Y. Laitman, P.A. Ganz, G.E. Tomlinson, S.L. Neuhausen, S.A. Narod, C.M. Phelan, R. Greenberg, K.L. Nathanson, Modification of BRCA1-Associated Breast and Ovarian Cancer Risk by BRCA1-Interacting Genes, Cancer Res. 71 (2011) 5792–5805, https://doi.org/10.1158/0008-5472.CAN-11-0773.
[88] S. Bonache, I. Esteban, A. Moles-Fernández, A. Tenés, L. Duran-Lozano, G. Montalban, V. Bach, E. Carrasco, N. Gadea, A. López-Fernández, S. Torres-Esquius, F. Mancuso, G. Caratú, A. Vivancos, N. Tuset, J. Balmaña, S. Gutiérrez-Enríquez, O. Diez, Multigene panel testing beyond BRCA1/2 in breast/ovarian cancer Spanish families and clinical actionability of findings, J Cancer Res Clin Oncol. 144 (2018) 2495–2513, https://doi.org/10.1007/s00432-018-2763-9.
[89] S. Vilkki, V. Launonen, A. Karhu, P. Sistonen, I. Västrik, L.A. Aaltonen, Screening for microsatellite instability target genes in colorectal cancers, Journal of Medical Genetics. 39 (2002) 785–789, https://doi.org/10.1136/jmg.39.11.785.
[90] C. Bilbao, R. Ramírez, G. Rodríguez, O. Falcón, L. León, N. Díaz-Chico, M. Perucho, J.C. Díaz-Chico, Double strand break repair components are frequent targets of microsatellite instability in endometrial cancer, European Journal of Cancer. 46 (2010) 2821–2827, https://doi.org/10.1016/j.ejca.2010.06.116.
[91] T.J. Gaymes, A.M. Mohamedali, M. Patterson, N. Matto, A. Smith, A. Kulasekararaj, R. Chelliah, N. Curtin, F. Farzaneh, S. Shall, G.J. Mufti, Microsatellite instability induced mutations in DNA repair genes CtIP and MRE11 confer hypersensitivity to poly (ADP-ribose) polymerase inhibitors in myeloid malignancies, Haematologica. 98 (2013) 1397–1406, https://doi.org/10.3324/haematol.2012.079251.
[92] G. Ciriello, E. Cerami, C. Sander, N. Schultz, Mutual exclusivity analysis identifies oncogenic network modules, Genome Research. 22 (2012) 398–406, https://doi.org/10.1101/gr.125567.111.
[93] R. Vélez-Cruz, D.G. Johnson, The Retinoblastoma (RB) Tumor Suppressor: Pushing Back against Genome Instability on Multiple Fronts, International Journal of Molecular Sciences. 18 (2017) 1776, https://doi.org/10.3390/ijms18081776.
[94] W. Zhang, Y. Song, X. He, X. Liu, Y. Zhang, Z. Yang, P. Yang, J. Wang, K. Hu, W. Liu, X. Zhang, X. Yuan, H. Jing, Prognosis value of RBBP8 expression in plasma cell myeloma, Cancer Gene Ther. (2019) 1–8, https://doi.org/10.1038/s41417-018-0069-3.
[95] J. Mijnes, J. Veeck, N.T. Gaisa, E. Burghardt, T.C. de Ruijter, S. Gostek, E. Dahl, D. Pfister, S.C. Schmid, R. Knüchel, M. Rose, Promoter methylation of DNA damage repair (DDR) genes in human tumor entities: RBBP8/CtIP is almost exclusively methylated in bladder cancer, Clin Epigenet. 10 (2018) 15, https://doi.org/10.1186/s13148-018-0447-6.
[96] Y. Yu, L. Chen, G. Zhao, H. Li, Q. Guo, S. Zhu, P. Li, L. Min, S. Zhang, RBBP8/CtIP suppresses P21 expression by interacting with CtBP and BRCA1 in gastric cancer, Oncogene (2019) 1–17, https://doi.org/10.1038/s41388-019-1060-7.
[97] M. Wu, D.R. Soler, M.C. Abba, M.I. Nunez, R. Baer, C. Hatzis, A. Llombart-Cussac, A. Llombart-Bosch, C.M. Aldaz, CtIP Silencing as a Novel Mechanism of Tamoxifen Resistance in Breast Cancer, Mol Cancer Res. 5 (2007) 1285–1295, https://doi.org/10.1158/1541-7786.MCR-07-0126.
[98] J. Wang, Q. Ding, H. Fujimori, A. Motegi, Y. Miki, M. Masutani, Loss of CtIP disturbs homologous recombination repair and sensitizes breast cancer cells to PARP inhibitors, Oncotarget 7 (2015) 7701–7714, https://doi.org/10.18632/oncotarget.6715.
[99] I. Soria-Bretones, C. Sáez, M. Ruíz-Borrego, M.A. Japón, P. Huertas, Prognostic value of CtIP/RBBP8 expression in breast cancer, Cancer Medicine. 2 (2013) 774–783, https://doi.org/10.1002/cam4.141.
[100] C.R. Reczek, R. Shakya, Y. Miteva, M. Szabolcs, T. Ludwig, R. Baer, The DNA resection protein CtIP promotes mammary tumorigenesis, Oncotarget 7 (2016) 32172–32183, https://doi.org/10.18632/oncotarget.8605.
[101] Y. Zhang, M. Jasin, An essential role for CtIP in chromosomal translocation formation through an alternative end-joining pathway, Nat Struct Mol Biol. 18 (2011) 80–84, https://doi.org/10.1038/nsmb.1940.
[102] M. Lee-Theilen, A.J. Matthews, D. Kelly, S. Zheng, J. Chaudhuri, CtIP promotes microhomology-mediated alternative end joining during class-switch recombination, Nat Struct Mol Biol. 18 (2011) 75–79, https://doi.org/10.1038/nsmb.1942.
[103] S. Badie, A.R. Carlos, C. Folio, K. Okamoto, P. Bouwman, J. Jonkers, M. Tarsounas, BRCA1 and CtIP promote alternative non-homologous end-joining at uncapped telomeres, The EMBO Journal 34 (2015) 410–424, https://doi.org/10.15252/embj.201488947.
[104] R. Bhargava, M. Fischer, R.J. O'Sullivan, Genome rearrangements associated with aberrant telomere maintenance, Current Opinion in Genetics & Development. 60 (2020) 31–40, https://doi.org/10.1016/j.gde.2020.02.005.
[105] J. Maciejowski, T. de Lange, Telomeres in cancer: tumour suppression and genome instability, Nat Rev Mol Cell Biol. 18 (2017) 175–186, https://doi.org/10.1038/nrm.2016.171.
[106] N.A. Willis, R.L. Frock, F. Menghi, E.E. Duffey, A. Panday, V. Camacho, E. P. Hasty, E.T. Liu, F.W. Alt, R. Scully, Mechanism of tandem duplication formation in BRCA1 -mutant cells, Nature 551 (2017) 590–595, https://doi.org/10.1038/nature24477.
[107] F. Menghi, F.P. Barthel, V. Yadav, M. Tang, B. Ji, Z. Tang, G.W. Carter, Y. Ruan, R. Scully, R.G.W. Verhaak, J. Jonkers, E.T. Liu, The Tandem Duplicator Phenotype Is a Prevalent Genome-Wide Cancer Configuration Driven by Distinct Gene Mutations, Cancer Cell. 34 (2018) 197–210, https://doi.org/10.1016/j.ccell.2018.06.008, e5.
[108] H. Knollman, J.L. Godwin, R. Jain, Y.-N. Wong, E.R. Plimack, D.M. Geynisman, Muscle-invasive urothelial bladder cancer: an update on systemic therapy,

Therapeutic Advances in Urology. (2015), https://doi.org/10.1177/1756287215607418.

[109] R. Torrisi, M. Zuradelli, E. Agostinetto, G. Masci, A. Losurdo, R. De Sanctis, A. Santoro, Platinum salts in the treatment of BRCA-associated breast cancer: A true targeted chemotherapy? Critical Reviews in Oncology/Hematology. 135 (2019) 66–75, https://doi.org/10.1016/j.critrevonc.2019.01.016.

[110] P.G. Pilié, C. Tang, G.B. Mills, T.A. Yap, State-of-the-art strategies for targeting the DNA damage response in cancer, Nat Rev Clin Oncol. 16 (2019) 81–104, https://doi.org/10.1038/s41571-018-0114-z.

[111] J.A. Nickoloff, D. Jones, S.-H. Lee, E.A. Williamson, R. Hromas, Drugging the Cancers Addicted to DNA Repair, JNCI: Journal of the National Cancer Institute. 109 (2017), https://doi.org/10.1093/jnci/djx059.

[112] A. Trenner, A.A. Sartori, Harnessing DNA Double-Strand Break Repair for Cancer Treatment, Front. Oncol. 9 (2019), https://doi.org/10.3389/fonc.2019.01388.

[113] W.G. Kaelin, The Concept of Synthetic Lethality in the Context of Anticancer Therapy, Nat Rev Cancer. 5 (2005) 689–698, https://doi.org/10.1038/nrc1691.

[114] M.Y.T. Keung, Y. Wu, J.V. Vadgama, PARP Inhibitors as a Therapeutic Agent for Homologous Recombination Deficiency in Breast Cancers, Journal of Clinical Medicine. 8 (2019) 435, https://doi.org/10.3390/jcm8040435.

[115] A. Min, S.-A. Im, PARP Inhibitors as Therapeutics: Beyond Modulation of PARylation, Cancers. 12 (2020) 394, https://doi.org/10.3390/cancers12020394.

[116] S.P. Ivy, J. de Bono, E.C. Kohn, The 'Pushmi-Pullyu' of DNA REPAIR: Clinical Synthetic Lethality, Trends in Cancer. 2 (2016) 646–656, https://doi.org/10.1016/j.trecan.2016.10.014.

[117] S.M. Noordermeer, H. van Attikum, PARP Inhibitor Resistance: A Tug-of-War in BRCA-Mutated Cells, Trends in Cell Biology. (2019), https://doi.org/10.1016/j.tcb.2019.07.008.

[118] E. Gogola, S. Rottenberg, J. Jonkers, Resistance to PARP Inhibitors: Lessons from Preclinical Models of BRCA-Associated Cancer, Annual Review of Cancer Biology. 3 (2019) 235–254, https://doi.org/10.1146/annurev-cancerbio-030617-050232.

[119] P. Francica, S. Rottenberg, Mechanisms of PARP inhibitor resistance in cancer and insights into the DNA damage response, Genome Medicine. 10 (2018) 101, https://doi.org/10.1186/s13073-018-0612-8.

[120] V. Kari, W.Y. Mansour, S.K. Raul, S.J. Baumgart, A. Mund, M. Grade, H. Sirma, R. Simon, H. Will, M. Dobbelstein, E. Dikomey, S.A. Johnsen, Loss of CHD1 causes DNA repair defects and enhances prostate cancer therapeutic responsiveness, EMBO Reports 17 (2016) 1609–1623, https://doi.org/10.15252/embr.201642352.

[121] R. Ceccaldi, J.C. Liu, R. Amunugama, I. Hajdu, B. Primack, M.I.R. Petalcorin, K. W. O'Connor, P.A. Konstantinopoulos, S.J. Elledge, S.J. Boulton, T. Yusufzai, A. D. D'Andrea, Homologous-recombination-deficient tumours are dependent on Polθ-mediated repair, Nature. 518 (2015) 258–262, https://doi.org/10.1038/nature14184.

[122] J. Schimmel, R. van Schendel, J.T. den Dunnen, M. Tijsterman, Templated Insertions: A Smoking Gun for Polymerase Theta-Mediated End Joining, Trends in Genetics. 35 (2019) 632–644, https://doi.org/10.1016/j.tig.2019.06.001.

[123] C. Sun, J. Yin, Y. Fang, J. Chen, K.J. Jeong, X. Chen, C.P. Vellano, Z. Ju, W. Zhao, D. Zhang, Y. Lu, F. Meric-Bernstam, T.A. Yap, M. Hattersley, M.J. O'Connor, H. Chen, S. Fawell, S.-Y. Lin, G. Peng, G.B. Mills, BRD4 Inhibition Is Synthetic Lethal with PARP Inhibitors through the Induction of Homologous Recombination Deficiency, Cancer Cell. 33 (2018) 401–416, https://doi.org/10.1016/j.ccell.2018.01.019, e8.

[124] Y. Duan, Y. Guan, W. Qin, X. Zhai, B. Yu, H. Liu, Targeting Brd4 for cancer therapy: inhibitors and degraders, Med. Chem. Commun. 9 (2018) 1779–1802, https://doi.org/10.1039/C8MD00198G.

[125] L. Shen, Q. Shi, W. Wang, Double agents: genes with both oncogenic and tumor-suppressor functions, Oncogenesis. 7 (2018) 1–14, https://doi.org/10.1038/s41389-018-0034-x.

[126] K.A. Hoadley, C. Yau, T. Hinoue, D.M. Wolf, A.J. Lazar, E. Drill, R. Shen, A. M. Taylor, A.D. Cherniack, V. Thorsson, R. Akbani, R. Bowlby, C.K. Wong, M. Wiznerowicz, F. Sanchez-Vega, A.G. Robertson, B.G. Schneider, M. S. Lawrence, H. Noushmehr, T.M. Malta, S.J. Caesar-Johnson, J.A. Demchok, I. Felau, M. Kasapi, M.L. Ferguson, C.M. Hutter, H.J. Sofia, R. Tarnuzzer, Z. Wang, L. Yang, J.C. Zenklusen, J. (Julia) Zhang, S. Chudamani, J. Liu, L. Lolla, R. Naresh, T. Pihl, Q. Sun, Y. Wan, Y. Wu, J. Cho, T. DeFreitas, S. Frazer, N. Gehlenborg, G. Getz, D.I. Heiman, J. Kim, M.S. Lawrence, P. Lin, S. Meier, M.S. Noble, G. Saksena, D. Voet, H. Zhang, B. Bernard, N. Chambwe, V. Dhankani, T. Knijnenburg, R. Kramer, K. Leinonen, Y. Liu, M. Miller, S. Reynolds, I. Shmulevich, V. Thorsson, W. Zhang, R. Akbani, B.M. Broom, A.M. Hegde, Z. Ju, R.S. Kanchi, A. Korkut, J. Li, H. Liang, S. Ling, W. Liu, Y. Lu, G.B. Mills, K.-S. Ng, A. Rao, M. Ryan, J. Wang, J.N. Weinstein, J. Zhang, A. Abeshouse, J. Armenia, D. Chakravarty, W.K. Chatila, I. de Bruijn, J. Gao, B.E. Gross, Z.J. Heins, R. Kundra, K. La, M. Ladanyi, A. Luna, M.G. Nissan, A. Ochoa, S.M. Phillips, E. Reznik, F. Sanchez-Vega, C. Sander, N. Schultz, R. Sheridan, S.O. Sumer, Y. Sun, B.S. Taylor, J. Wang, H. Zhang, P. Anur, M. Peto, P. Spellman, C. Benz, J. M. Stuart, C.K. Wong, C. Yau, D.N. Hayes, J.S. Parker, M.D. Wilkerson, A. Ally, M. Balasundaram, R. Bowlby, D. Brooks, R. Carlsen, E. Chuah, N. Dhalla, R. Holt, S.J.M. Jones, K. Kasaian, D. Lee, Y. Ma, M.A. Marra, M. Mayo, R.A. Moore, A. J. Mungall, K. Mungall, A.G. Robertson, S. Sadeghi, J.E. Schein, P. Sipahimalani, A. Tam, N. Thiessen, K. Tse, T. Wong, A.C. Berger, R. Beroukhim, A.D. Cherniack, C. Cibulskis, S.B. Gabriel, G.F. Gao, G. Ha, M. Meyerson, S.E. Schumacher, J. Shih, M.H. Kucherlapati, R.S. Kucherlapati, S. Baylin, L. Cope, L. Danilova, M. S. Bootwalla, P.H. Lai, D.T. Maglinte, D.J.V.D. Berg, D.J. Weisenberger, J. T. Auman, S. Balu, T. Bodenheimer, C. Fan, K.A. Hoadley, A.P. Hoyle, S. R. Jefferys, C.D. Jones, S. Meng, P.A. Mieczkowski, L.E. Mose, A.H. Perou, C. M. Perou, J. Roach, Y. Shi, J.V. Simons, T. Skelly, M.G. Soloway, D. Tan, U. Veluvolu, H. Fan, T. Hinoue, P.W. Laird, H. Shen, W. Zhou, M. Bellair, K. Chang, K. Covington, C.J. Creighton, H. Dinh, H. Doddapaneni, L. A. Donehower, J. Drummond, R.A. Gibbs, R. Glenn, W. Hale, Y. Han, J. Hu, V. Korchina, S. Lee, L. Lewis, W. Li, X. Liu, M. Morgan, D. Morton, D. Muzny, J. Santibanez, M. Sheth, E. Shinbrot, L. Wang, M. Wang, D.A. Wheeler, L. Xi, F. Zhao, J. Hess, E.L. Appelbaum, M. Bailey, M.G. Cordes, L. Ding, C.C. Fronick, L. A. Fulton, R.S. Fulton, C. Kandoth, E.R. Mardis, M.D. McLellan, C.A. Miller, H. K. Schmidt, R.K. Wilson, D. Crain, E. Curley, J. Gardner, K. Lau, D. Mallery, S. Morris, J. Paulauskis, R. Penny, C. Shelton, T. Shelton, M. Sherman, E. Thompson, P. Yena, J. Bowen, J.M. Gastier-Foster, M. Gerken, K.M. Leraas, T. M. Lichtenberg, N.C. Ramirez, L. Wise, E. Zmuda, N. Corcoran, T. Costello, C. Hovens, A.L. Carvalho, A.C. de Carvalho, J.H. Fregnani, A. Longatto-Filho, R. M. Reis, C. Scapulatempo-Neto, H.C.S. Silveira, D.O. Vidal, A. Burnette, J. Eschbacher, B. Hermes, A. Noss, R. Singh, M.L. Anderson, P.D. Castro, M. Ittmann, D. Huntsman, B. Kohl, X. Le, R. Thorp, C. Andry, E.R. Duffy, V. Lyadov, O. Paklina, G. Setdikova, A. Shabunin, M. Tavobilov, C. McPherson, R. Warnick, R. Berkowitz, D. Cramer, C. Feltmate, N. Horowitz, A. Kibel, M. Muto, C.P. Raut, A. Malykh, J.S. Barnholtz-Sloan, W. Barrett, K. Devine, J. Fulop, Q. T. Ostrom, K. Shimmel, Y. Wolinsky, A.E. Sloan, A.D. Rose, F. Giuliante, M. Goodman, B.Y. Karlan, C.H. Hagedorn, J. Eckman, J. Harr, J. Myers, K. Tucker, L.A. Zach, B. Deyarmin, H. Hu, L. Kvecher, C. Larson, R.J. Mural, S. Somiari, A. Vicha, T. Zelinka, J. Bennett, M. Iacocca, B. Rabeno, P. Swanson, M. Latour, L. Lacombe, B. Têtu, A. Bergeron, M. McGraw, S.M. Staugaitis, J. Chabot, H. Hibshoosh, A. Sepulveda, T. Su, T. Wang, O. Potapova, O. Voronina, L. Desjardins, O. Mariani, S. Roman-Roman, X. Sastre, M.-H. Stern, F. Cheng, S. Signoretti, A. Berchuck, D. Bigner, E. Lipp, J. Marks, S. McCall, R. McLendon, A. Secord, A. Sharp, M. Behera, D.J. Brat, A. Chen, K. Delman, S. Force, F. Khuri, K. Magliocca, S. Maithel, J.J. Olson, T. Owonikoko, A. Pickens, S. Ramalingam, D. M. Shin, G. Sica, E.G.V. Meir, H. Zhang, W. Eijckenboom, A. Gillis, E. Korpershoek, L. Looijenga, W. Oosterhuis, H. Stoop, K.E. van Kessel, E. C. Zwarthoff, C. Calatozzolo, L. Cuppini, S. Cuzzubbo, F. DiMeco, G. Finocchiaro, L. Mattei, A. Perin, B. Pollo, C. Chen, J. Houck, P. Lohavanichbutr, A. Hartmann, C. Stoehr, R. Stoehr, H. Taubert, S. Wach, B. Wullich, W. Kycler, D. Murawa, M. Wiznerowicz, K. Chung, W.J. Edenfield, J. Martin, E. Baudin, G. Bubley, R. Bueno, A.D. Rienzo, W.G. Richards, S. Kalkanis, T. Mikkelsen, H. Noushmehr, L. Scarpace, N. Girard, M. Aymerich, E. Campo, E. Giné, A.L. Guillermo, N. V. Bang, P.T. Hanh, B.D. Phu, Y. Tang, H. Colman, K. Evason, P.R. Dottino, J. A. Martignetti, H. Gabra, H. Juhl, T. Akeredolu, S. Stepa, D. Hoon, K. Ahn, K. J. Kang, F. Beuschlein, A. Breggia, M. Birrer, D. Bell, M. Borad, A.H. Bryce, E. Castle, V. Chandan, J. Cheville, J.A. Copland, M. Farnell, T. Flotte, N. Giama, T. Ho, M. Kendrick, J.-P. Kocher, K. Kopp, C. Moser, D. Nagorney, D. O'Brien, B. P. O'Neill, T. Patel, G. Petersen, F. Que, M. Rivera, L. Roberts, R. Smallridge, T. Smyrk, M. Stanton, R.H. Thompson, M. Torbenson, J.D. Yang, L. Zhang, F. Brimo, J.A. Ajani, A.M.A. Gonzalez, C. Behrens, O. Bondaruk, R. Broaddus, B. Czerniak, B. Esmaeli, J. Fujimoto, J. Gershenwald, C. Guo, A.J. Lazar, C. Logothetis, F. Meric-Bernstam, C. Moran, L. Ramondetta, D. Rice, A. Sood, P. Tamboli, T. Thompson, P. Troncoso, A. Tsao, I. Wistuba, C. Carter, L. Haydu, P. Hersey, V. Jakrot, H. Kakavand, R. Kefford, K. Lee, G. Long, G. Mann, M. Quinn, R. Saw, R. Scolyer, K. Shannon, A. Spillane, J. Stretch, M. Synott, J. Thompson, J. Wilmott, H. Al-Ahmadie, T.A. Chan, R. Ghossein, A. Gopalan, D. A. Levine, V. Reuter, S. Singer, B. Singh, N.V. Tien, T. Broudy, C. Mirsaidi, P. Nair, P. Drwiega, J. Miller, J. Smith, H. Zaren, J.-W. Park, N.P. Hung, E. Kebebew, W. M. Linehan, A.R. Metwalli, K. Pacak, P.A. Pinto, M. Schiffman, L.S. Schmidt, C. D. Vocke, N. Wentzensen, R. Worrell, H. Yang, M. Moncrieff, C. Goparaju, J. Melamed, H. Pass, N. Botnariuc, I. Caraman, M. Cernat, I. Chemencedji, A. Clipca, S. Doruc, G. Gorincioi, S. Mura, M. Pirtac, I. Stancul, D. Tcaciuc, M. Albert, I. Alexopoulou, A. Arnaout, J. Bartlett, J. Engel, S. Gilbert, J. Parfitt, H. Sekhon, G. Thomas, D.M. Rassl, R.C. Rintoul, C. Bifulco, R. Tamakawa, W. Urba, N. Hayward, H. Timmers, A. Antenucci, F. Facciolo, G. Grazi, M. Marino, R. Merola, R. de Krijger, A.-P. Gimenez-Roqueplo, A. Piché, S. Chevalier, G. McKercher, K. Birsoy, G. Barnett, C. Brewer, C. Farver, T. Naska, N.A. Pennell, D. Raymond, C. Schilero, K. Smolenski, F. Williams, C. Morrison, J.A. Borgia, M. J. Liptay, M. Pool, C.W. Seder, K. Junker, L. Omberg, M. Dinkin, G. Manikhas, D. Alvaro, M.C. Bragazzi, V. Cardinale, G. Carpino, E. Gaudio, D. Chesla, S. Cottingham, M. Dubina, F. Moiseenko, R. Dhanasekaran, K.-F. Becker, K.-P. Janssen, J. Slotta-Huspenina, M.H. Abdel-Rahman, D. Aziz, S. Bell, C. M. Cebulla, A. Davis, R. Duell, J.B. Elder, J. Hilty, B. Kumar, J. Lang, N. L. Lehman, R. Mandt, P. Nguyen, R. Pilarski, K. Rai, L. Schoenfield, K. Senecal, P. Wakely, P. Hansen, R. Lechan, J. Powers, A. Tischler, W.E. Grizzle, K.C. Sexton, A. Kastl, J. Henderson, S. Porten, J. Waldmann, M. Fassnacht, S.L. Asa, D. Schadendorf, M. Couce, M. Graefen, H. Huland, G. Sauter, T. Schlomm, R. Simon, P. Tennstedt, O. Olabode, M. Nelson, O. Bathe, P.R. Carroll, J.M. Chan, P. Disaia, P. Glenn, R.K. Kelley, C.N. Landen, J. Phillips, M. Prados, J. Simko, K. Smith-McCune, S. VandenBerg, K. Roggin, A. Fehrenbach, A. Kendler, S. Sifri, R. Steele, A. Jimeno, F. Carey, I. Forgie, M. Mannelli, M. Carney, B. Hernandez, B. Campos, C. Herold-Mende, C. Jungk, A. Unterberg, A. von Deimling, A. Bossler, J. Galbraith, L. Jacobus, M. Knudson, T. Knutson, D. Ma, M. Milhem, R. Sigmund, A.K. Godwin, R. Madan, H.G. Rosenthal, C. Adebamowo, S.N. Adebamowo, A. Boussioutas, D. Beer, T. Giordano, A.-M. Mes-Masson, F. Saad, T. Bocklage, L. Landrum, R. Mannel, K. Moore, K. Moxley, R. Postier, J. Walker, R. Zuna, M. Feldman, F. Valdivieso, R. Dhir, J. Luketich, E.M.M. Pinero, M. Quintero-Aguilo, C.G. Carlotti, J.S.D. Santos, R. Kemp, A. Sankarankuty, D. Tirapelli, J. Catto, K. Agnew, E. Swisher, J. Creaney, B. Robinson, C.S. Shelley, E. M. Godwin, S. Kendall, C. Shipman, C. Bradford, T. Carey, A. Haddad, J. Moyer, L. Peterson, M. Prince, L. Rozek, G. Wolf, R. Bowman, K.M. Fong, I. Yang, R. Korst, W.K. Rathmell, J.L. Fantacone-Campbell, J.A. Hooke, A.J. Kovatich, C.

D. Shriver, J. DiPersio, B. Drake, R. Govindan, S. Heath, T. Ley, B.V. Tine, P. Westervelt, M.A. Rubin, J.I. Lee, N.D. Aredes, A. Mariamidze, J.M. Stuart, C. C. Benz, P.W. Laird, Cell-of-Origin Patterns Dominate the Molecular Classification of 10,000 Tumors from 33 Types of Cancer, Cell 173 (2018) 291–304, https://doi.org/10.1016/j.cell.2018.03.022, e6.

[127] A.K. Witkiewicz, E.A. McMillan, U. Balaji, G. Baek, W.-C. Lin, J. Mansour, M. Mollaee, K.-U. Wagner, P. Koduru, A. Yopp, M.A. Choti, C.J. Yeo, P. McCue, M. A. White, E.S. Knudsen, Whole-exome sequencing of pancreatic cancer defines genetic diversity and therapeutic targets, Nat Commun. 6 (2015) 1–11, https://doi.org/10.1038/ncomms7744.

[128] M. Goldman, B. Craft, M. Hastie, K. Repečka, F. McDade, A. Kamath, A. Banerjee, Y. Luo, D. Rogers, A.N. Brooks, J. Zhu, D. Haussler, The UCSC Xena platform for public and private cancer genomics data visualization and interpretation, BioRxiv (2019), 326470, https://doi.org/10.1101/326470.